COMMUNICATIONS TO THE EDITOR

Inhibition of Ribonucleotide Reductase Activity by IC202C

Sir:

In the course of our screening program for low molecular weight immunomodulators, we found new hydroxamate siderophores, IC202A, B and C, produced by *Streptoalloteichus* sp. 1454-19, which have structurally characteristics in their terminal moieties such as butyliden *N*-oxide, nitro and aldoxime groups, respectively.[1~3] Among them, IC202C (Fig. 1) is a major product and shows the strongest immunosuppressive activity.[3] In this communication, we report the effect of IC202C against ribonucleotide reductase activity.

It is known that deferoxamine, a ferrioxamine compound, which also has a hydroxamate moiety like IC202C, suppresses DNA synthesis by inhibition of ribonucleotide reductase.[4] Ribonucleotide reductase plays an essential role in DNA synthesis in most organisms.[5] The enzyme is a nonheme iron-containing protein, which requires ATP and Mg^{2+} as cofactors. As previously reported that IC202C has an affinity against Fe(III) ion,[3] we have examined the effect of IC202C on ribonucleotide reductase activity using EL4 mouse T lymphoma cells. EL4 cell (7×10^7) extracts were prepared as described by HOFFBRAND *et al.*,[4] and ribonucleotide reductase activity in the cell extracts was determined according to the method by THELANDAR[6] and GLEASON,[5] using [5-^{3}H]cytidine 5′-diphoshate ammonium salt as a substrate. After the reaction at 37°C for 1 hour, the radioactivity of dCMP was counted by a liquid scintillation counter.

As a result, IC202C significantly inhibited ribonucleotide reductase in EL4 cell extracts *in vitro* as well as hydroxyurea and deferoxamine (Sigma-Aldrich Japan, Tokyo), typical inhibitors of ribonucleotide reductase (Table 1). IC202C also inhibited the enzyme activity in concanavalin A-treated mouse splenocytes *in vitro* (control; 0.71 nmol/mg protein, IC202C 50 μg/ml; 0.32 nmol/mg protein). Thus, IC202C was found to be a new inhibitor of ribonucleotide reductase. Because IC202C associates Fe(III) ion, it might inactivate the enzyme through scavenging the ion or directly binding to the ion held in the enzyme. Indeed, our preliminary data showed that IC202C pre-complexed with Fe(III) ion lost the inhibitory activity on the enzyme. Although the precise mechanism of immunosuppressive activity of IC202C is still unknown, inhibition of ribonucleotide reductase activity is considered to be major cause for suppression of T cell proliferation in MLCR by IC202C.

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Fig. 1. Structure of IC202C.

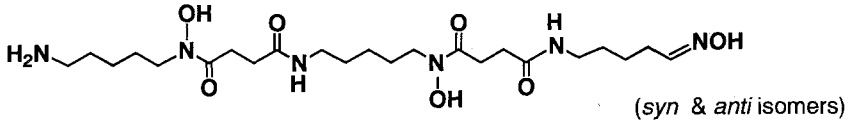

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Table 1. Effect of IC202C on ribonucleotide reductase activity in EL4 cells.

sample	(μg/ml)	Ribonucleotide reductase (nmol/mg protein)
	Exp. 1	
saline		8.7
IC202C	5	4.4
	50	2.7
	500	2.3
	Exp. 2	
saline		13.3
IC202C	50	1.3
deferoxamine	50	2.9
hydroxyurea	50	3.2

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